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Exacerbation of rat adjuvant arthritis by intradermal injection of purified mammalian 14-kDa group II phospholipase A_2

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Appreciable phospholipase A_2 activity was detected in a hind paw homogenate from rats with adjuvant arthritis or carrageenan-induced edema. The activity was neutralized by treatment with antibody raised against rat platelet secretory phospholipase A_2, indicating that 14-kDa group II phospholipase A_2 was induced in the hind paw during the process of inflammation. Injection of purified rat platelet phospholipase A_2 into the hind paw of rats with adjuvant-induced arthritis resulted in exacerbation of edema in a dose-dependent manner, whereas no effect was observed on either normal rats or animals with carrageenan-induced edema under the same experimental conditions. These results suggest the importance of mammalian group II phospholipase A_2 in the pathogenesis of some types of inflammation.

Phospholipase A_2; Adjuvant arthritis; Carrageenan-induced edema

1. INTRODUCTION

Phospholipase A_2 plays a central role in liberating free fatty acids and lysophospholipids from membrane phospholipids, thereby initiating the production of lipid mediators (eicosanoids, platelet activating factor, etc.) of the inflammatory process. Several studies have implicated extracellular phospholipases A_2 in the pathogenesis of disorders of the cardiovascular, gastrointestinal and pulmonary systems, skin and connective tissues [1]. Mammalian 14-kDa phospholipases A_2 have been classified into two groups based on their primary structure [2]. Group I enzymes exist mainly in digestive organs such as the pancreas [3,4], while group II enzymes are widely distributed in non-pancreatic tissues such as the spleen [5,6], liver [7], intestine [8] or platelets [9–11]. The group II enzymes have also been detected in exudated fluid at inflamed sites, such as glycogen-induced ascitic fluid in rabbits [12], casein-induced ascitic fluid in rats [13], or human synovial fluid in patients with rheumatoid arthritis [14–16]. It is therefore postulated that group II phospholipase A_2 may play some critical role in the process of inflammation. This idea has been further supported by observations that inflammation was induced in animals by injection of phospholipases A_2 [12–21]. However, such induction of inflammation has not always been observed, and sometimes no appreciable change has been detected at sites where purufied enzyme was injected.

When a rat receives an intradermal injection of complete Freund's adjuvant, chronic arthritis with remarkable paw edema develops, and this has been widely used as a model system for human rheumatoid arthritis. In the present study, we found that the levvel of phospholipase A_2 was greatly elevated under these pathological conditions. Also, we observed that injection of mammalian 14-kDa group II phospholipase A_2 purified from rat platelets exacerbated the paw edema in rats with adjuvant arthritis. It is suggested that some pathological conditions may be required in order for the enzyme to become active.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediamine tetraacetate; PBS, phosphate-buffered saline, SDA-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Adjuvant arthritis*

Male rats of the Sprague-Dawley (SD) strain, weighing 190–200 g (8 weeks old), were used. The adjuvant employed, a fine suspension of dry heat-killed *Mycobacterium butyricum* (Difco) in light mineral oil (Bayol F), was made up at a concentration of 10 mg/ml. Adjuvant arthritis was induced by an intradermal injection of 0.06 ml of adjuvant into the tail. Two weeks after the injection of adjuvant, rats showing medium to severe arthritis with remarkable edema (approximately 90% of total population) were selected and used [22].

2.2. *Carrageenan-induced hind paw edema in rats*

Male SD rats, weighing 100–130 g, were used. Hind paw edema was

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induced by subcutaneous injection of 0.1 ml of 1% carrageenan (Wako) solution into the right foot pad of rats [23].

2.3. *Measurement of phospholipase A_2 activity in hind paw homogenates*

Normal rats or those with inflammation were anesthetized with ether and killed. The hind paws of these rats were minced with scissors, suspended in 4 vol of 20 mM Tris-HCl (pH 7.4) containing 1 mM EDTA relative to wet tissue weight, and homogenized in a Polytron homogenizer (Kinematica). Phospholipase A_2 activity in the homogenates was measured using ^{14}C-labelled phosphatidylserine as a substrate, as described previously [24].

2.4. *Measurement of phospholipase A_2 content by sandwich ELISA*

Group II phospholipase A_2 content in hind paw homogenates was determined by sandwich ELISA using solid-phase attached polyclonal antibody R377 and the soluble biotinylated monoclonal antibody MD7.1 as described previously [25]. Immunoaffinity-purified rat platelet phospholipase A_2 was used as a standard.

3. RESULTS

The homogenates of hind paw obtained from normal rats, or those with adjuvant-induced arthritis or carrageenan-induced edema were tested for phospholipase A_2 activity. Appreciable phospholipase A_2 activity was detected in homogenates prepared from hind paws with both arthritis and carrageenan-induced paw edema, whereas the level of enzyme activity in the same parts of normal rat was very low (Fig. 1). When the homogenates were incubated with anti-rat 14-kDa group II phospholipase A_2 antibody R377 [25], the phospholipase A_2 activity was markedly reduced (Table I). The content of group II phospholipase A_2 in the homogenates was determined immunochemically using a sandwich ELISA method developed previously (Table II). Approximately 1 μg of group II phospholipase A_2 was detected in the inflamed site of rat with adjuvant arthritis. The hind paw of rats with carrageenan-induced edema also contained a significantly high level of group II phospholipase A_2, although the content was relatively lower than that in adjuvant arthritis. It was thus concluded that mammalian 14-kDa group II phospholipase A_2 is induced in both types of inflammation.

We next examined the effect of exogenous group II phospholipase A_2 on the development of inflammation induced by either adjuvant-arthritic rats or rats with carrageenan-induced edema. To avoid the possibility that contaminating substances exerted biological effects, we used the purest preparation so far available, an immunoaffinity-purified rat platelet phospholipase A_2, which gave a single protein band on SDS-PAGE followed by silver staining [25]. Injection of the phospholipase A_2 into the hind paw of adjuvant-arthritic rats resulted in exacerbation of edema within 1 h in a dose-dependent manner (Table III). The maximum effect was observed at doses of over 2 μg of enzyme. The effect lasted for at least 3 h, then decreased gradually, and disappeared 24 h after injection of the enzyme. On the other hand, no exacerbating effect was observed in either rats with carrageenan-induced edema or control rats even when 5 μg of phospholipase A_2 was injected (Tables IV and V).

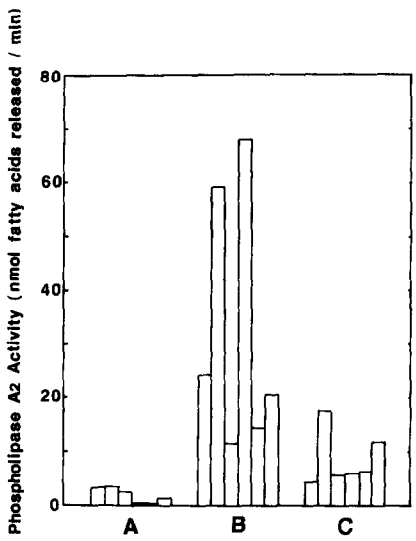

Fig. 1. Phospholipase A_2 activity in hind paw homogenates from normal rats (A), rats with adjuvant arthritis (B) and rats with carrageenan-induced edema (C) (n = 6). The procedure is described in section 2.

Table I

Effect of anti-rat 14-dKa group II phospholipase A_2 antibody on phospholipase A_2 activity in rat hind paw homogenates

Sample	Treatment with R377	Phospholipase A_2 activity (nmol) fatty acids released/min/ml
Normal	–	0.63 ± 0.20
	+	0.41 ± 0.70
Adjuvant arthritis	–	5.04 ± 0.56
	+	0.96 ± 0.14
Carrageenan-induced edema	–	3.45 ± 1.17
	+	0.70 ± 0.22

Samples were incubated with 25 μg of antibody R377 [25] for 1 h on ice. The aliquots were then examined for phospholipase A_2 activity. Each value is an average (± SE) of 3 independent samples.

Table II

Content of 14-kDa group II phospholipase A_2 in hind paw homogenates determined by sandwich ELISA

Sample	*n*	Content of phospholipase A_2 (average (ng) ± SE)
Normal	6	not detectable
Adjuvant arthritis	6	932.6 ± 691.2
Carrageenan-induced edema	6	136.8 ± 34.1

Content of phospholipase A_2 was determined as described previously [25].

Table III
Effect of 14-kDa group II phospholipase A_2 on adjuvant induced arthritis in rats

Dose of phospholipase A_2 (μg/paw)	n	Hind paw swelling (average (%) ± SE)			
		1	3	6	24 h
0 (untreated control)	6	0.8 ± 1.8	4.3 ± 1.6	3.2 ± 1.3	6.2 ± 2.7
0 (vehicle control)	6	9.5 ± 2.0	10.6 ± 1.3	8.8 ± 1.2	4.2 ± 3.7
0.2	6	11.4 ± 4.3	11.7 ± 5.0	7.8 ± 4.3	−1.1 ± 1.8
1	6	15.2 ± 2.4	13.1 ± 2.4	9.5 ± 2.4	4.7 ± 1.1
2	6	18.4 ± 2.4*	17.0 ± 3.5	15.6 ± 3.2	8.7 ± 2.2
5	6	16.6 ± 2.2*	17.2 ± 1.9*	14.5 ± 1.6*	6.4 ± 3.4

Purified rat platelet phospholipase A_2 was diluted with phosphate-buffered saline (PBS; pH 6.5) containing 0.1% gelatin and injected into the hind paw of rats with adjuvant arthritis (0.1 ml/paw). Hind paw volume was measured at indicated times after the injection of the enzyme by the water displacement method [22,23].
* $0.01 < P < 0.05$ significantly different from each vehicle group

Table IV
Effect of 14-kDa group II phospholipase A_2 on carrageenan-induced hind paw edema in rats

Dose of phospholipase A_2 (μg/paw)	n	Hind paw swelling (average (%) ± SE)				
		1	2	3	4	5 h
0 (untreated control)	6	4.4 ± 1.0*	1.4 ± 0.8**	−0.6 ± 1.9*	−4.5 ± 2.1*	−8.3 ± 2.1*
0 (vehicle control)	6	8.3 ± 1.0	6.9 ± 0.6	6.3 ± 0.5	2.9 ± 0.7	−2.2 ± 1.2
2	6	7.1 ± 0.7	7.2 ± 0.8	5.5 ± 1.2	1.1 ± 1.3	−2.1 ± 1.3
5	6	6.2 ± 0.7	4.9 ± 0.8	3.1 ± 0.9**	−0.8 ± 1.2*	−5.8 ± 0.9

Two hours after injection of carrageenan, purified rat platelet phospholipase A_2 in PBS (pH 6.5) containing 0.1% gelatin was injected into the hind paw of rats with edema (0.1 ml/paw). Hind paw volume was measured as described in Table III.
* $0.01 < P < 0.05$ and ** $P < 0.01$ significantly different from each vehicle control group

Table V
Effect of 14-kDa group II phospholipase A_2 on normal rats

Dose of phospholipase A_2 (μg/paw)	n	Hind paw swelling (average (%) ± SE)				
		1	2	3	4	5 h
0 (untreated control)	6	1.9 ± 2.0**	0.4 ± 1.3**	−0.3 ± 1.0**	−3.7 ± 1.2**	−0.7 ± 1.3**
0 (vehicle control)	6	17.8 ± 2.4	14.4 ± 1.7	12.1 ± 1.5	6.4 ± 1.8	1.5 ± 0.6
5	6	15.2 ± 0.9	8.1 ± 1.4*	6.8 ± 1.5*	1.3 ± 1.4	−1.1 ± 1.4

Purified rat platelet phospholipase A_2 in PBS (pH 6.5) containing 0.1% gelatin was injected into the hind paw of normal rats (0.1 ml/paw). Hind paw volume was measured as described in Table III.
* $0.01 < P < 0.05$ and ** $P < 0.01$ significantly different from each vehicle control group

4. DISCUSSION

Adjuvant arthritis in rats is characterized as a form of chronic inflammation mediated by cellular immunity [26], whereas carrageenan-induced edema is an acute inflammation showing predominant infiltration of neutrophils [27]. In the present study, we detected an elevated level of 14-kDa group II phospholipase A_2 activity at the inflamed sites in both experimental models, although the location of the phospholipase A_2 remains to be identified. The results were consistent with previous observations that 14-kDa group II phospholipase A_2 activity was detected in extra-articular tissues under various pathological conditions, which included experimental models of acute inflammation and human chronic inflammatory diseases [12–16]. These observations, together with a recent report that a potent regulator of inflammation, interleukin-1, induced transcription of the gene for 14-kDa group II phospholipase A_2 [28], suggest the possible involvement of the enzyme in a wide variety of pathogenic reactions.

Purified rat group II phospholipase A_2 exacerbated the adjuvant arthritis following injection in situ. The effect was transient and induced at low doses of the enzyme. The lowest amount of purified enzyme required for an appreciable effect was 1 μg, which corresponds to about 20 units of the enzyme. Several investigators have previously pointed out the pathological activity of snake venom or pancreatic phospholipases A_2 when administered exogenously. However, the amounts of these exocrine enzymes used were extraordinarily high

[17–20]; Pruzanski et al. showed that inflammatory effects of snake venom or pancreatic phospholipase A_2 were induced by injection of 60 000 units of enzyme [17]. The amount of the enzyme we used in the present study was within the range of activity detected in the hind paw homogenates of rats with experimental inflammation. Exogenously administered 14-kDa group II phospholipase A_2 produced a dose-dependent effect. The endogenous 14-kDa group II phospholipase A_2 would in fact exacerbate adjuvant arthritis after secretion into the extracellular space under certain conditions. The precise mechanism by which 14-kDa group II phospholipase A_2 exacerbates the inflammatory process is now under investigation.

No appreciable inflammatory response was detected when 14-kDa group II phospholipase A_2 was injected into the hind paw of normal rats. These findings were contradictory to previous reports of others.

Vishwanath et al. have shown that phospholipase A_2 partially purified from human rheumatoid synovial fluid induced edema when injected into mouse foot pads [21]. Phospholipase A_2 released from rabbit peritoneal exudate cells induced hyperemia subsequent to intradermal injection [19]. The discrepancy between the previous findings and the present data might be due to differences in sensitivity of the species employed. Alternatively, since only partially purified enzyme was used in previous studies, such preparations might have contained additional component(s) that affected the activity of the 14-kDa group II phospholipase A_2 and potentiated the induction of some pathological responses. In fact, one such component, a bacterial permeability increasing protein, has already been characterized by Elsbach et al. [29].

It is noteworthy that no exacerbating effect was observed in rats with carrageenan-induced edema when the animals were injected with 5 μg (100 units) of the phospholipase A_2. The difference in the effects of exogenous phospholipase A_2 on adjuvant arthritis and carrageenan-induced edema cannot be explained at present. The major cellular component in carrageenan-induced edema is neutrophils, whereas that in adjuvant arthritis is mononuclear cells such as T lymphocytes or macrophages. Some cells accompanying with arthritis might therefore release factor(s) essential for expression of the pathological effects of mammalian 14-kDa group II phospholipase A_2.

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